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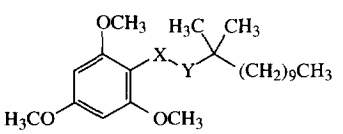
INHIBITORS OF ACYL-CoA: CHOLESTEROL *O*-ACYL TRANSFERASE (ACAT) AS HYPOCHOLESTEROLEMIC AGENTS. 12. SYNTHESES AND BIOLOGICAL ACTIVITY OF STRUCTURALLY NOVEL TETRAZOLE AMIDES.

Patrick M. O'Brien*†, Drago R. Sliskovic†, Maureen K. Anderson, Richard F. Bousley, Brian R. Krause, and Richard L. Stanfield

Departments of Medicinal Chemistry† and Atherosclerosis Therapeutics, Parke-Davis Pharmaceutical Research, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105.

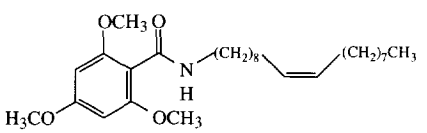
Abstract: The identification of tetrazole benzamide and nicotinamide derivatives as new structural classes of potent ACAT inhibitors is described. The ensuing structure-activity relationship (SAR) studies revealed that retroamide **8c** and **7q** possesses comparable in vitro potency and efficacy to that of the fatty acid anilide, CI-976 (**1**).

ACAT catalyzes the intracellular esterification of cholesterol in most mammalian tissues by using CoA-activated fatty acids to produce cholesteryl ester.[1] Considerable experimental evidence suggests that the ACAT-mediated esterification of cholesterol plays a key role in intestinal sterol absorption, hepatic production of very low density lipoproteins (VLDL),[2] and the unregulated accumulation of cholesteryl esters in atherosclerotic lesions.[3] Thus, inhibition of ACAT may be of therapeutic value for individuals with elevated levels of plasma cholesterol by reducing the amount of cholesterol absorbed from the intestine, and/or by reducing stores of hepatic cholesteryl esters necessary for VLDL synthesis and secretion.[4] Furthermore, systemically available agents may prevent the accumulation of cholesteryl ester-rich foam cells within the arterial wall, thereby arresting lesion development at an early stage in the atherogenic process.



(**1**) X=NH, Y=CO

(**2**) X=CO, Y=NH



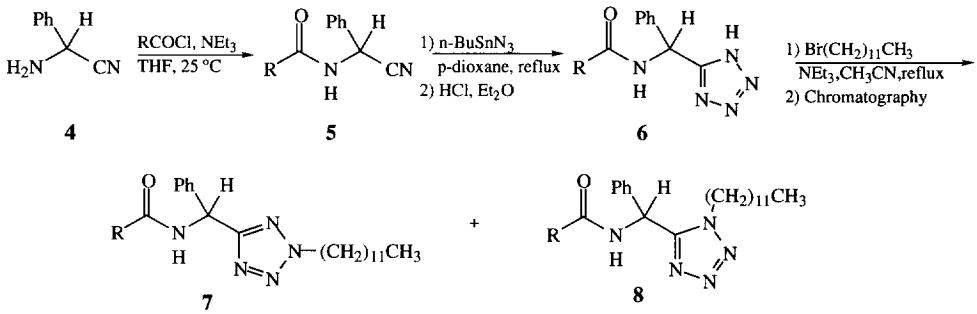
(**3**)

We have previously reported on a series of fatty acid anilides that are potent ACAT inhibitors *in vitro* and efficacious hypocholesterolemic agents *in vivo*.[5] Evidence has been provided that one member of this series, CI-976 (**1**), inhibits the progression and can enhance the regression of atherosclerotic lesions in cholesterol-fed rabbits, with or without a reduction in plasma total cholesterol.[6] Subsequent bioisosteric replacement of the anilide moiety, revealed that retroamides **2**[7] and **3**,[8] were significantly less active (IC_{50}'s >10 μM and 9.4 μM respectively) when compared

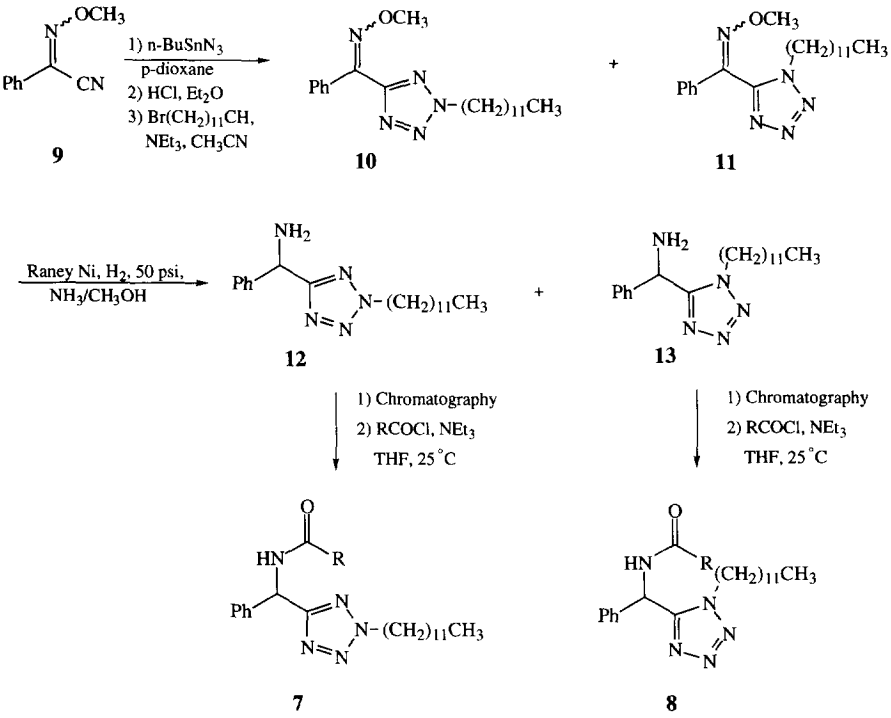
to the corresponding anilide isomers (IC_{50}'s= 0.110 μM and 0.050 μM respectively). Further studies involving the bioisosteric replacement of the amide moiety identified a series of *N*-2,6-diisopropylphenyl-*N'*-alkylureas that were shown to potently inhibit ACAT *in vitro* with IC_{50}'s in the 20 to 60 nM range.[8] The ensuing SAR studies of these secondary ureas examined the affect of replacing the aliphatic chain with structurally diverse functionalities.[9,10,11,12] Among these, a series of alkylated tetrazoles were shown to be extremely potent *in vitro* and efficacious *in vivo*.[12] Thus, in an attempt to improve the potency of the retroamides previously described, we decided to investigate the effects of incorporation of the tetrazole moiety into the side chain of the α - substituted retroamides. Utilizing 2-phenylglycinonitrile (**4**), we synthesised a novel series of retroamide tetrazole derivatives (Scheme 1) which, unlike **2** and **3**, exhibited potency comparable to **1**. Herein we describe the synthesis and biological activity of this novel series of ACAT inhibitors.

Chemistry: The synthetic route used to prepare compounds **7a,c,e** and **8e** is illustrated in Scheme 1. The amides **5a,c,e** were synthesized by acylating (±)-2-phenylglycinonitrile (**4**) with the appropriate acid chloride in the presence of triethylamine (60-80%). The resulting α–amidonitriles **5a,c,e** were converted to the corresponding tetrazole derivatives **6a,c,e** by treatment with tributyltin azide in refluxing dioxane, with subsequent cleavage of tributyltin from the tetrazole moiety with ethereal HCl (80-85%). Alkylation of **6a,c,e** with 1-bromododecane provided a 3:1 mixture of regioisomers **7** and **8**,[13] from which pure **7a,c,e** and **8e** could be isolated by silica gel chromatography, albeit in low yield (10-40%).

SCHEME 1



SCHEME 2



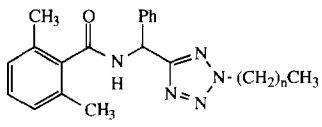
Due to the difficult chromatographic separation, the corresponding 1-regioisomers of **7a,c** were not isolated at this time and an alternate synthetic approach (Scheme 2) was used to prepare the remaining compounds employed in this study. The tetrazole intermediates **10** and **11** were prepared by the cycloaddition of tributyltin azide with **9** (57%), followed by alkylation of the resulting tetrazole to give a 1:1 mixture of regioisomers (80%). Hydrogenation of the oxime ether moiety using Raney nickel in methanolic ammonia afforded the penultimate amines **12** (32%) and **13** (26%), which were isolated isomerically pure by silica gel chromatography. These were then converted to amides **7b,d,f-q** and **8c,d,k,o,q** (80-90%) by treatment with the appropriate acid chloride in the presence of triethylamine.

Biological Methods: The ability of compounds to inhibit ACAT activity was measured *in vitro* by incubating test compounds with [1-^{14}C]oleoyl-CoA and microsomes isolated from the livers of cholesterol-fed rats.[14] For potent inhibitors, hypocholesterolemic activity was assessed in rats given a single dose of test compound (30 mg/kg) by gavage suspended in carboxymethyl-cellulose

(CMC) and Tween-20 in water, followed by a single high-fat, high-cholesterol meal. The following day, total serum cholesterol levels were determined and the data expressed as percent decrease relative to controls.[14]

Results and Discussion: The initial compound prepared for this study, **7c**, was significantly more potent *in vitro* and efficacious *in vivo* (40% decrease in plasma total cholesterol) than the fatty alkyl benzamides **2** and **3** (IC_{50}= 0.31 μM v.s >10 and 9.4 μM respectively). To evaluate the role of the tetrazole side chain, we prepared the C_6 (**7r**) and C_{14} (**7s**) analogs of **7c** (Table I). These changes significantly diminished inhibitory activity, thus complimenting the tetrazole urea SAR, which demonstrated that the dodecyl side chain is within the chain length range (C_{10}-C_{14}) necessary for potent inhibition.[12] The positioning of the alkyl side chain on the tetrazole ring was also examined (Table II). Unlike the tetrazole ureas, the 1-regioisomer **8c**, was more potent and efficacious than the 2-regioisomer **7c**. The opposite effect was observed for nicotinamide derivatives, **7q** and **8q**. In general, the positioning of the tetrazole alkyl chain is not critical for potent inhibition in this series.

TABLE I

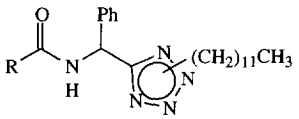


Compd	n	IC_{50} (μM)[a]	% Change TC[d]
7c	12	0.11	-40*
7r	6	3.70	-13
7s	14	0.44	-14

[a]ACAT inhibition in vitro, intestinal microsomes isolated from cholesterol-fed rabbits. See reference 14 for complete protocol.
[b] See footnote d in Table II.

We then directed our efforts toward examining the SAR for substitution on the benzamide ring. The results (Table II) show that substituents previously reported to provide optimal activity for the anilide and urea ACAT inhibitors,[15] did not enhance biological activity.
Thus, replacement of the 2,6-dimethyl moiety of **7c** with 2,6-diisopropyl (**7b**), 2,6-dichloro (**7d**), or 2,4,6-trimethoxy (**7a**) failed to increase potency or efficacy. Somewhat suprisingly, the unsubstituted derivative **7f**, was essentially equipotent to **7c** *in vitro,* although it was not as efficacious *in vivo*. In order to improve the potency for these inhibitors, compounds **7g-k** were

TABLE II



Compd	R	Formula[a]	Isomer	mp°C[b]	IC_{50} (μM)[c]	% Change TC[d]
1	CI 976				0.11	-73*
2	CI 976 (retroamide)	$C_{23}H_{39}NO_4$		oil	>10	-10
3		$C_{28}H_{47}NO_4$		wax	9.40	NT[e]
7a	2,4,6-$(CH_3O)_3$Ph	$C_{30}H_{43}N_5O_4$	2	74-75	1.70	-36*
7b	2,6-iPr_2Ph	$C_{33}H_{49}N_5O$	2	80-81	0.81	+2
7c	2,6-$(CH_3)_2$Ph	$C_{29}H_{41}N_5O$	2	51-52	0.31	-40*
8c	2,6-$(CH_3)_2$Ph	$C_{29}H_{41}N_5O$	1	85-87	0.18	-57*
7d	2,6-Cl_2Ph	$C_{27}H_{35}Cl_2N_5O$	2	102-103	0.46	-26*
8d	2,6-Cl_2Ph	$C_{27}H_{35}Cl_2N_5O$	1	124-125	0.38	-31*
7e	2,4-F_2Ph	$C_{27}H_{35}F_2N_5O$	2	57-58	0.49	-10
8e	2,4-F_2Ph	$C_{27}H_{35}F_2N_5O$	1	88-90	0.38	-23*
7f	Ph	$C_{27}H_{37}N_5O$	2	79-80	0.35	-5
7g	4-ClPh	$C_{27}H_{36}ClN_5O$	2	89-90	0.44	NT[e]
7h	4-CH_3OPh	$C_{28}H_{39}N_5O_2$	2	94-95	0.60	-34*
7i	4-CH_3Ph	$C_{28}H_{39}N_5O$	2	94-95	0.51	-4
7j	3-ClPh	$C_{27}H_{36}ClN_5O$	2	71-73	0.25	+3
7k	3-NO_2Ph	$C_{27}H_{36}N_6O_3$	2	83-84	0.08	-33*
8k	3-NO_2Ph	$C_{27}H_{36}N_6O3$	1	84-85	0.19	-31*
7l	2-NO_2Ph	$C_{27}H_{36}N_6O_3$	2	97-99	0.24	-27*
7m	4-NO_2Ph	$C_{27}H_{36}N_6O_3$	2	83-84	0.54	+8
7n	4-CF_3Ph	$C_{28}H_{36}F_3N_5O$	2	100-102	0.85	-10
7o	2-CF_3Ph	$C_{28}H_{36}F_3N_5O$	2	69-71	0.12	-15*
8o	2-CF_3Ph	$C_{28}H_{36}F_3N_5O$	1	120-121	0.35	-12
7p	2-CH_3OPh	$C_{28}H_{39}N_5O_2$	2	oil	0.65	-28*
7q	2-CH_3SPyr	$C_{27}H_{38}N_6OS$	2	70-72	0.08	-68*
8q	2-CH_3SPyr	$C_{27}H_{38}N_6O_5$	1	151-152	0.14	-37*

[a] Analytical results are within ± 0.4% of the theoretical values. [b] Melting points are uncorrected. [c] ACAT inhibition in vitro, liver microsomes isolated from cholesterol-fed rats. Each determination performed in triplicate. See reference 14 for complete protocol. [d] Denotes percent change in total cholesterol in cholic acid (0.5%)-cholesterol (1.5%)-peanut oil (5.5%)-fed rats. See reference 14 for the complete protocol. [e] Not tested. * Denotes significantly different from control, $p < 0.05$ using analysis of variance followed by Fisher's multiple range test.

prepared using the Topliss scheme for aromatic substituent selection.[16] As predicted, optimal potency was obtained for the 3-nitrobenzamide derivative, **7k**, having an IC_{50} of 0.08 μM. Expecting further improvements in *in vitro* activity, the 1-regioisomer **8k** was prepared. However, unlike **8c**, **8k** is 2 fold less active than the corresponding 2-regioisomer **7k**. Despite having comparable potency *in vitro*, **7k** was not as effective as **1** *in vivo*.

In an effort to improve aqueous solubility and possibly absorption properties for this series, the benzamide ring was replaced with a substituted nicotinamide moiety. Replacing the aryl ring gave **7q**, a potent ACAT inhibitor (IC_{50}= 0.08 μM) which was equiefficacious compared to **1** and considerably more efficacious than the benzamides. The 1-regioisomer **8q**, however, was nearly 2-fold less potent than **7q**.

In summary, we have examined the structure-activity relationship for a series of retroamide tetrazole derivatives, focusing primarily on substituents attached to the benzamide

moiety. For these compounds, the length of the tetrazole side chain (C_{12}) was crucial for potent ACAT inhibition, whereas its position (i.e. 1 v.s 2 regioisomers) was less critical. Of the substituents evaluated on the benzamide ring, the 3-nitro derivative **7k**, provided optimal activity *in vitro*, but the 2,6-dimethyl substituted compound, **8c**, was considerably more efficacious *in vivo*. Compound **7q** however, was equipotent to **7k** *in vitro* but more efficacious *in vivo*, and comparable to **8c** and **1**, in the cholesterol-fed rat model. This may have been due to the pyridine nitrogen atom which provides a site of protonation and thus may improve solubility and absorption properties. Further extensions of this study will be the topic of future communications from these laboritories.

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